CHROMOSOME PAIRING IN OBLIGATELY APOGAMOUS FERNS; PELLAEA ATROPURPUREA AND PELLAEA GLABELLA VAR. GLABELLA

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Obligate apogamy is a type of asexual reproduction, which is found in several genera of ferns, including *Pellaea*, the cliff brakes. Among the species of this genus which show obligate apogamy are two which can be found in north-eastern North America, namely *Pellaea atropurpurea* (L.) Link and *P. glabella* Mett. ex Kuhn. As part of a more general investigation of these two species (Rigby 1968) cytological studies were made on their sporangia during sporogenesis.

In obligately apogamous ferns the sporophyte arises directly from the gametophyte without fertilization, while viable spores are still produced by meiosis as in sexually reproducing ferns. Therefore, a compensating mechanism is necessary to keep the chromosome number from being repeatedly reduced by meiosis from one generation to the next. Such a mechanism was observed by Steil (1919) and described in detail by Manton (1950). In the final mitotic division before meiosis the chromosomes of the spore mother cells divide, but the cells themselves do not, so that restitution nuclei are formed. Thus, instead of the sporangium containing sixteen spore mother cells each with the same chromosome number as the sporophyte plant, the sporangium contains only eight spore mother cells, each with double the sporophytic chromosome number. These spore mother cells can then go through a regular meiosis, producing thirty-two viable spores, each with the same number of chromosomes as the sporophyte.

This type of compensating mechanism does not occur in all the sporangia of the apogamous plant. Of the four types of sporangia found by Manton (1950) in varying proportions in the apogamous ferns she investigated, the two which seem to be most useful for cytogenetic studies are:

Type One — There is no premeiotic doubling of the chromosome number. Sixteen spore mother cells are formed and meiosis begins, but many of the chromosomes do not pair. This lack of pairing and irregular disjunction leads to spore abortion.

Type Two — The premeiotic doubling of the chromosome number gives eight spore mother cells, in each of which meiosis can take place with regular pairing between the newly doubled chromosomes. Thirty-two viable spores are produced.

Type Two sporangia, with their regular bivalents, can be used to determine the chromosome number of the species. In the Type One sporangia the chromosomes may not consist of two homologous sets, for pairing is irregular. Since many apogamous ferns are triploid or of a higher ploidy level and may owe their origin to hybridization, Manton (1950) suggests that the degree of homology between the genomes of the original parents of the cross may be deduced from examination of the pairing patterns in Type One sporangia.

Pellaea atropurpurea is an apogamous triploid, and the most common form of *P. glabella* var. *glabella* (the eastern variety of this species) is an apogamous tetraploid. The chromosome numbers of these two taxa have been established as 87 for *P. atropurpurea* and 116 for *P. glabella* var. *glabella* (Manton 1950; Tryon & Britton 1958). During the present study Type One sporangia of the two taxa were examined to ascertain, as far as possible, the relative proportions of univalents, bivalents and multivalents present in each. It was thought that these pairing patterns might give some indication as to the origin of these taxa, that is, whether they had arisen as autopolyploids or had begun as hybrids between two or more species.

MATERIALS AND METHODS

The sources of material used for studying the meiotic chromosomes were as follows:

Pellaea atropurpurea (L.) Link

(i) Spore-grown specimen. Source of spores, collection by *A. Monette,* Campbell's Bay, Quebec (DAO 26361).

(ii) Spore-grown specimen. Source of spores, collection by *T. M. C. Taylor,* Fairmount Hotsprings, British Columbia (UBC 37640).

P. glabella Mett. ex Kuhn var. *glabella*

(i) Spore-grown specimen. Source of spores, collection by *J. B. Clark,* Laclede Co., Missouri (OAC 37706).

(ii) Material collected in the field, by *S. J. Rigby,* Rockwood, Ontario.

Fertile pinnae were fixed in 3 parts absolute ethanol: 1 part glacial acetic acid (previously saturated with ferric acetate) for 3 to 8 days at 4°C and then transferred to 70% ethanol and stored at 4°C until examined. Sporangial squashes were made using the propionic-iron-haematoxylin technique described by Lu (1967), with the modification that Solution A was diluted with about an equal volume of 45% acetic acid, since otherwise there was a tendency for the iron to precipitate when the two solutions were mixed. Slides were made permanent by ringing the cover slip with Hoyer's mounting medium (formula given in Alexopoulos & Beneke 1952, p. 2). The chromosomes were observed and photographed under interference contrast (Zeiss-Nomarski) with an oil immersion objective (Planapo, N.A. 1.3). Because the chromosomes in the Type One spore mother cells do not separate readily at metaphase, it was hoped that the three dimensional effect of the interference contrast might make it easier to distinguish between two or more overlapping chromosomal associations. In cases where the cells could not be flattened into a single focal plane, photographs were taken of the same cell in several focal planes. Tracings from these photographs were compared with the original slides and used to make the explanatory diagrams.

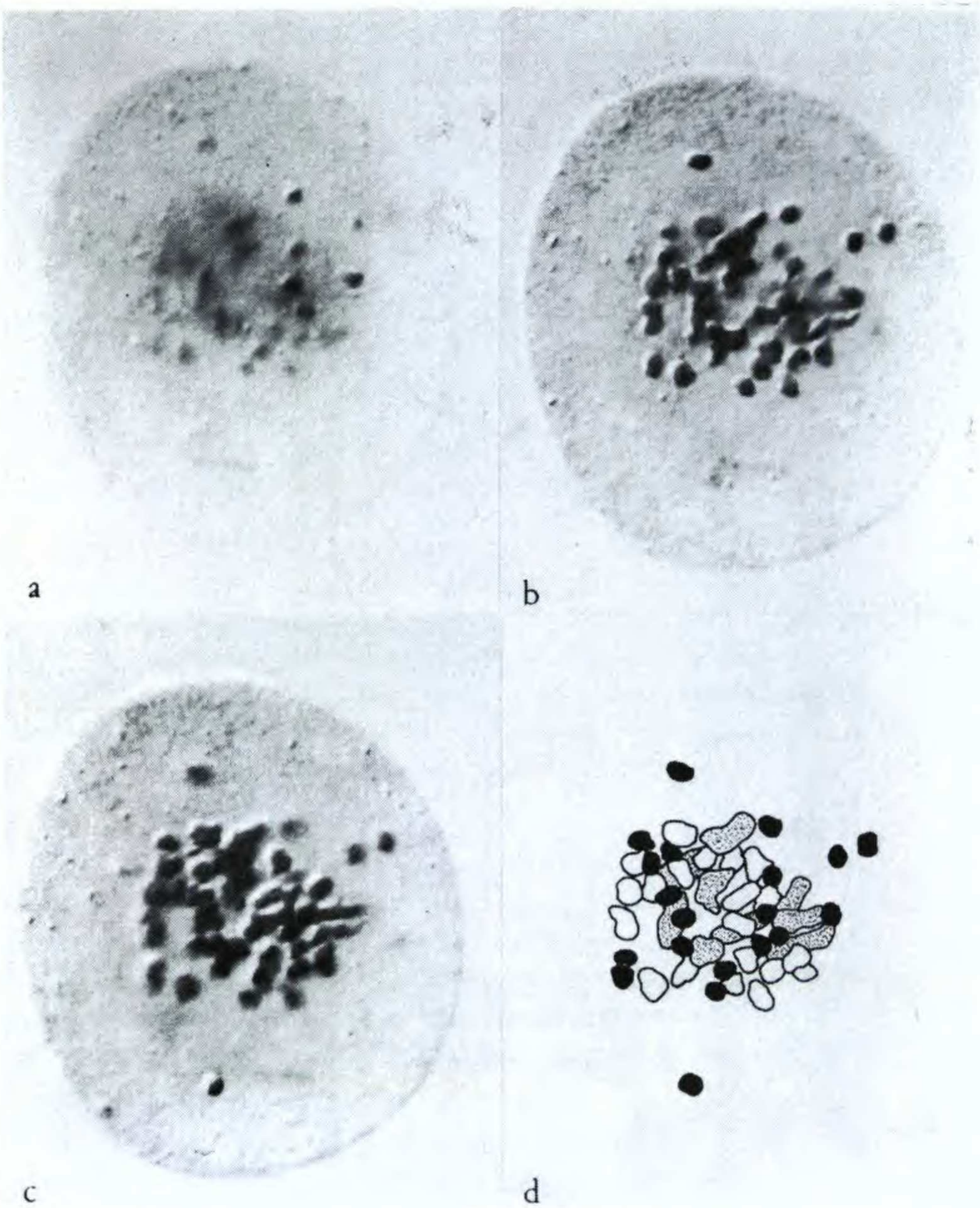

Figure 1. Spore mother cell from Type One sporangium of *Pellaea atropurpurea* (Campbell's Bay material) at meiosis. × 2000. a, b, c: Cell photographed in three different focal planes. d: Explanatory diagram, Interpretation: Univalents (black) 20, Bivalents (white) 20, Trivalents (stippled) 9.

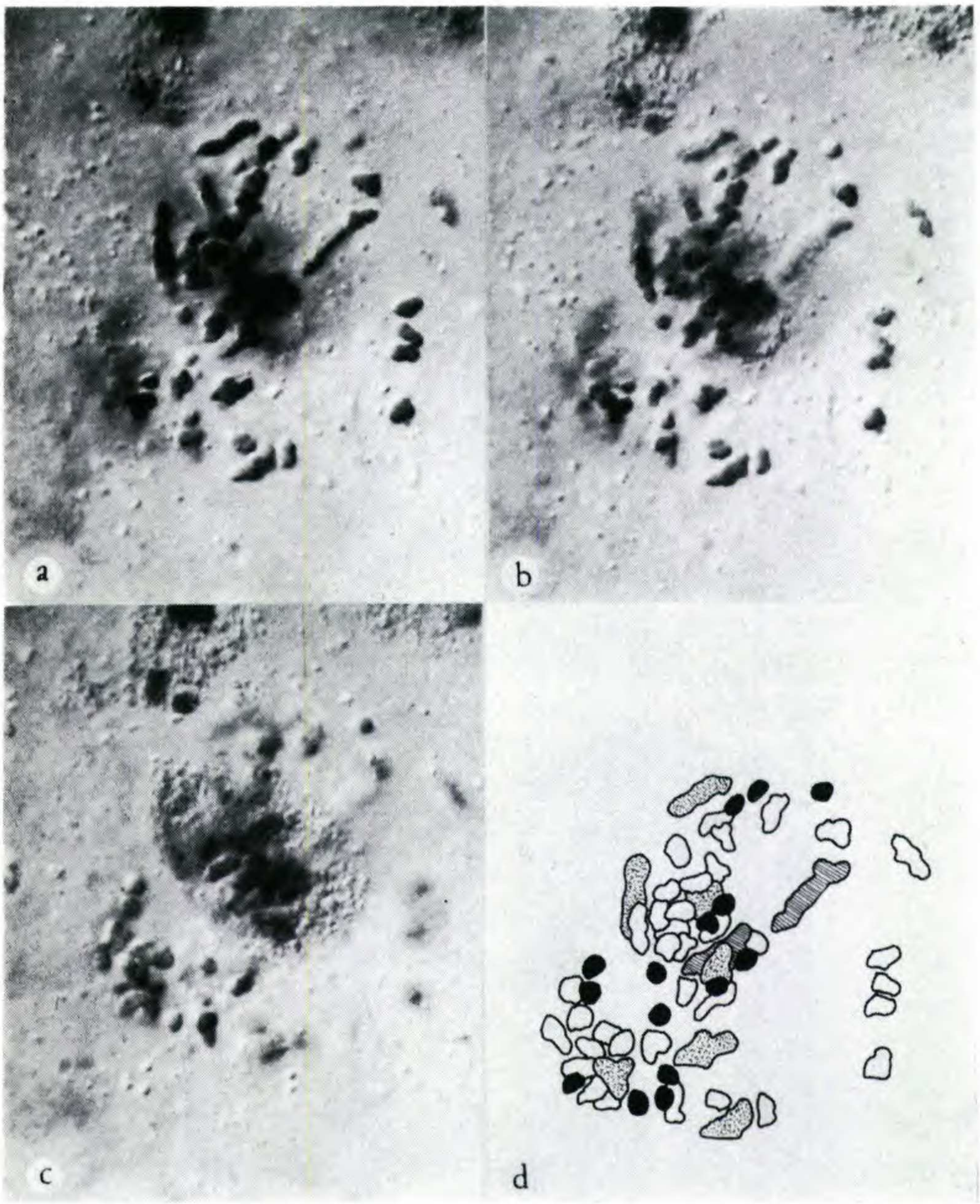

Figure 2. Spore mother cell from Type One sporangium of *Pellaea glabella* var. *glabella* (Laclede Co. material) at meiosis. × 2000. (Cell ruptured by pressure.) a, b, c: Cell photographed in three different focal planes. d: Explanatory diagram, Interpretation: Univalents (black) 15, Bivalents (white) 36, Trivalents (stippled) 7, Quadrivalents (hatched) 2.

Table 1.

Chromosomal association (univalent I to quadrivalent IV) frequencies in spore mother cells of Type One sporangia of *Pellaea*.

Taxon	Source of Material	I	II	III	IV
P. atropurpurea	Campbell's Bay, Quebec	20	20	9	
	Campbell's Bay	23	23	6	
	Fairmount Hotsprings, British Columbia	22	31	1	
P. glabella var. *glabella*	Rockwood, Ontario	34	20	10	3
	Rockwood	34	26	6	3
	Rockwood	39	18	11	2
	Rockwood	36	19	10	3
	Laclede Co., Missouri	26	32	6	2
	Laclede Co.	15	36	7	2

OBSERVATIONS

The chromosomes of the Type One spore mother cells tend to remain clumped together at meiosis and do not spread out well when squashed. Nine Type One cells were found that spread enough to be suitable for analysis, three from *P. atropurpurea* and six from *P. glabella* var. *glabella*. Photographs and explanatory diagrams of one cell from each of these taxa are shown in Figures 1 and 2, and the chromosomal association frequencies are summarized in Table 1. Even with the limited amount of material studied, it appears that in both taxa at least two thirds of the chromosomes are involved in some kind of association. Bivalents are more common than the higher order multivalents, but the relative frequencies of bivalents and univalents in the two samples of *P. glabella* var *glabella* differ; in the Rockwood material the univalents outnumber the bivalents, while in the material from Laclede County the proportions are reversed. In the Campbell's Bay material of *P. atropurpurea* the frequencies of univalents, bivalents and trivalents can be related to the basic chromosome number of 29, typical of the genus, but the material from Fairmount Hotsprings shows more than 29 associations.

DISCUSSION

Although the sample of Type One spore mother cells was not large enough for a meaningful statistical analysis, even within this small sample certain trends are indicated. In both species the proportions of different types of chromosomal associations show some variation from one population to another. In *P. atropurpurea* the material from Campbell's Bay suggests three sets of chromosomes, of which two sets possess a high degree of homology to each other, as shown by the formation of bivalents, while the third set has a lesser degree of homology to the other two, so that only about a quarter of its chromosomes go into the formation of trivalents, with the remainder being present as univalents. This type of chromosomal association might

indicate that *P. atropurpurea* arose from a cross between two distinct, though related species, one of which contributed two sets of chromosomes to the hybrid while the other contributed only one set. However, the cell from the Fairmount Hotsprings material, with its 31 bivalents, does not fit in with this pattern but rather suggests a more complex type of relationship.

In *P. glabella* var. *glabella* several probable quadrivalents were seen in the Type One spore mother cells from both populations, although their total proportion was low (about 10%). The presence of these quadrivalents suggests at least some homology among the four sets of chromosomes which originally contributed to the chromosome complement of this tetraploid. If quadrivalents, trivalents and bivalents are all cosidered, then on the average about 73% of the chromosomes in each cell are involved in some kind of chromosomal association, which again would suggest a fairly high degree of homology among these chromosome sets. This seems to indicate that *P. glabella* var. *glabella* originated either as an autopolyploid or from a cross between two closely related taxa. Because the species complex *P. glabella* includes two morphologically distinguishable, sexually reproducing diploid entities, namely var. *occidentalis* (E. Nelson) Butters, found in northwestern North America, and a diploid form of var. *glabella*, which so far has been reported only from Missouri (Wagner *et al.* 1965), possible ancestors for the tetraploid form of var. *glabella* can be found within the species complex.

While the patterns of choromosomal association observed in this study may suggest certain degrees of homology between the chromosome sets within each polyploid, it should be borne in mind that the degree of homology among the original sets of chromosomes of each polyploid may not be the only factor affecting chromosome pairing. Neither *P. atropurpurea* nor *P. glabella* var. *glabella* is a newly formed hybrid (or autopolyploid) but rather each has been reproducing asexually for many generations. During

this time changes may have taken place in the chromosome structure and in the genetic factors which govern chromosome pairing, so that the chromosomal associations seen at meiosis in the Type One spore mother cells are no longer fully indicative of the degree of homology between the chromosome sets involved. An indication that changes have taken place since these polyploids were first formed is the variation in chromosomal association frequencies between populations within each taxon. In fact, as Wagner (1963, 1970) points out, even in newly formed hybrids pairing may be influenced by genetic factors and therefore may not be a precise indicator of the degree of chromosome homology. The cytological observations made in this study cannot therefore be regarded as conclusive proof of the amount of homology between the various chromosome sets of the polyploids, but could serve as useful supporting evidence when used in conjunction with other types of observation, such as morphological characteristics.

SUMMARY

In obligately apogamous ferns the chromosome number is kept constant from generation to generation by a premeiotic doubling of the chromosome complement in some (but not all) of the spore mother cells, which then go through meiosis to produce viable spores. Spore mother cells in which such premeiotic doubling does not take place have irregular chromosome pairing at meiosis.

Pellaea atropurpurea is an apogamous triploid, while the most common form of *P. glabella* var. *glabella* is an apogamous tetraploid. In spore mother cells of these two polyploids — in which the chromosome number had not doubled when the chromosomes were observed at meiosis — univalents, bivalents and multivalents were all seen to be present. The patterns of chromosomal association observed could be interpreted as indicating homologies between the chromosome sets of the original parents of these polyploids, or they may be, at least in part, due to changes in chromosome structure and in the genetic factors governing

pairing that have taken place through many generations of asexual reproduction.

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